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O. Rotaru

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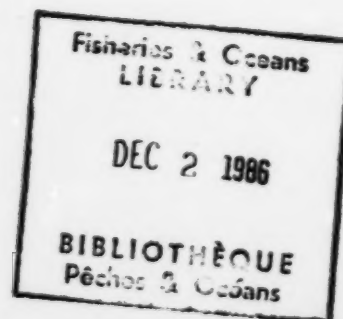
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EVALUATION OF METHODS FOR DIAGNOSING MYXOSOMA CEREBRALISINFECTION OF SALMONIDAE

By

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(From: Revista de Cresterera Animalelor, No. 8, p. 44-48, 1980; Animal Breeding Magazine, No. 8, p. 44-48, 1980)

Myxosoma cerebralis infection (caused by a sporozoan - Myxosoma cerebralis, of the family Myxosomatidae) was first recorded at the beginning of this century (Hoffer, 1903); in the last few decades it has shown increased incidence due to intensive worldwide breeding of salmonids (1, 2, 4, 7). The increase in the numbers of this highly productive species (rainbow trout - Salmo gairdnerii) which is very receptive to this parasitosis, is unfortunately offset by the large losses (up to 70%) through mortality. The disease attacks young trout (measuring up to 7 cm), during the first year of life, and the infestation coincides with the onset of the feeding period (13, 14).

The epizootological factors involved in the occurrence and spread of this disease gave impetus to intensified research on the biology of this parasite, improvement of diagnostic methods, and identification of effective means of prophylaxis and control. The absence of precise information on the incidence of this disease in our country, under conditions of intensive trout breeding, led us to direct long-term systematic research towards evaluation of current methods of diagnosis. This work reports the results of research on the validity, rapidity and efficiency of some methods for diagnosing Myxosoma cerebralis infection.

The investigations on paraclinical diagnosis were conducted on the following material: spawn, alevins, young fishes of different ages, adult trout, samples of water and silt taken from 12 trout-breeding basins and the 4 waterways connected to them. Checks were made repeatedly, as a function of the biology of the parasite and the technique used in trout-breeding in the period between 1976 and 1980. For purposes of obtaining trout samples for examination, material was classified on the basis of clinical and epizootologic criteria; samples of spawn, water and silt were also collected from places which were presumably contaminated.

Several methods were used to arrive at a diagnosis, with a view to determining their comparative value. Thus, direct microscopic examination was used on the water samples containing the spawn and on the total triturate of spawn and alevins, with direct examination of the ground, centrifuged cartilage from the levels of the skull and spinal column of young trout from which the soft tissues were removed; the

direct examination was carried out following the Lukcy method, which consists in revealing the zones selected by the parasite (Fig. 1). The surfaces of the cartilage were scraped with a scalpel and the scraped material examined between slides. Tests were conducted by using methods of enrichment through flotation (Willis, Daring) and centrifugation of triturate of the head and spinal column of young fish and silt coming from the trout breeding basins. The samples were analyzed after being colored with methylene blue or Lugol solutions. The sediment obtained from centrifugation of triturate of young fish treated by the gastric digestion method, was examined microscopically. There was also a histopathologic examination of the tissue obtained at the level of the skull, especially from the region close to the otic capsules, and from segments of the spinal column of young fishes (suspect and healthy) with clinical symptoms of disease. Hematoxylin-eosine, and trichrome-Masson methods were used for the staining of the section; at the same time, an X-ray examination was performed on specimens of adult trout with ossified skeletons. (45)

The results of the paraclinical examinations carried out using the above methods reveal several aspects linked to the speed and accuracy of diagnosis of Myxosoma cerebralis infection.

In the direct examination of the samples of water and spawn, the aim was to reveal the spores carried by water or found on the surface of the spawn, since transovarian transmission is impossible (2, 4, b). The results of the examinations revealed the presence of Myxosoma cerebralis spores, both at the level of the skull and the level of the spinal column.



Fig. 1: Longitudinal section through the skull of a young trout revealing the zones selected by Myxosoma cerebralis x 100.

The presence of Myxosoma cerebralis spores was also revealed through direct examination of cartilages of the skull and spinal column. These spores are lenticular in shape on a normal (perpendicular) plane, with a suture line, a diameter of 6.5-7x7.5-8 microns and a thickness of approximately 4 microns. The spore is composed of two polar capsules measuring 2.5-3x1.5-2 microns each, in which there are two spiral filaments 40-50 microns long and provided with micropyles. Inside, there is an ameboid embryo and the sporoplasm does not contain an iodophile vacuole; this absence differentiates this spore from those found in Myxobolus (4b, 5, 6, 7).

The speed and accuracy of this method are noteworthy; however, it has a disadvantage in that its results are belated in terms of the course of the disease, since the spore is a sign of tenacity and widespread attack by the disease, which consequently is discovered only in the advanced stages (1, 8a and b, 10). The silt samples were examined using methods of enrichment through flotation and centrifugation. However, this examination is difficult and laborious in view of the large quantities of material required and the need for special equipment, and it is of limited value in revealing the presence of spores. The results of the examination of ground samples and sediment enriched through centrifugation and flotation show that it cannot compare with the direct method used for the identification of spores of Myxosoma cerebralis, because of the time needed to process the samples and also because of the limited chances of detection. (46)

The results obtained with the use of the Lukcy method are conclusive in average and massive infestations, because the method is fast and easy to use.

The gastric digestion method recommended by different authors (3, 5, 9, 11, 12) is among the methods known for their accuracy in diagnosing the disease and is also valid in slight infestations; on the other hand, it requires complex laboratory techniques and takes a longer time to execute.

The histopathological examination permits detection and identification of the different stages of the parasite: trophozoid (Fig. 2), sporoblast, mature sporoblast (Fig. 3), pre-spore and spore. This fact permits diagnosis of the disease long before its symptoms become evident.

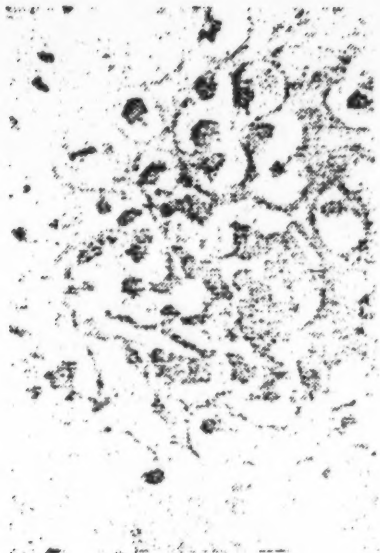


Fig. 2: Trophozooids x 900



Fig. 3: Mature spore x 900

The picture of the microlesions specific to Myxosoma cerebralis infection is characterized by the appearance of micro-foci of chondrolysis, with incipient tumefaction and destruction of chondrocytes. The invaded area is characterized by massive chondrocytic necrosis and penetration of trophozooids into the cartilage (Fig. 4). Large foci consist of extended zones of liquefaction (Fig. 5); in the peripheral region, the cartilage shows an anfractuous line of lysis, characterized by various types of chondrocytic alterations as a result of invasion by the parasite. In the periphery of the large foci, one notes a lympho-histiocytic infiltrative process (Fig. 6), marked in the chronic stages by the formation of ossified granulomas.

An X-ray examination was done in order to detect ossified parasitic granulomas in surviving adult trout which are possible carriers of spores of Myxosoma cerebralis.



Fig. 4: Chondrocytic necrosis caused by penetration of the cartilage by the trophozooid x 900



Fig. 5: Large zones of liquefaction x 200



Fig. 6: Reaction of the organism through the appearance of a lympho-histiocytic infiltrative process around foci x 100

CONCLUSIONS:

The fast, accurate diagnosis of massive and average infestations of Myxosoma cerebralis can be achieved by means of direct methods; enrichment and artificial digestion methods are effective in average and mild infestations.

The histopathological diagnosis reveals all the stages of the parasite and its specific group of microlesions, and consequently, permits early detection of the disease.

The X-ray examination is also valuable as a diagnostic tool, in that it detects adult trout which are carriers of parasitic granulomas.

Effective diagnosis of Myxosoma cerebralis infection requires the corroboration of preclinical information by clinical and epizootological data.

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